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Repeatable microbubble generation from nano-precursor for medical application

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Abstract

Proof of concept experiments on an novel ultrasonic diagnostic and therapeutic agent, phase change nano droplet (PCND), were performed. It was found using gel phantoms that PCND works as a cavitation accelerator only when ultrasound pulses which trigger the phase change of PCND from liquid to gas are exposed in advance. Another kind of nano droplet that cannot change its phase to a gas does not work even in the presence of the trigger. The cavitation induction with the aid of PCND was significant in B-mode echography as a brightness enhancement. Such a brightness change was also observed in in vivo experiments on tumor baring mice in the presence of systemically administered PCNDs and triggering pulses. Moreover, damages in tumor tissues were confirmed at the site of the brightness change. The lack of either a PCND or a phase change ultrasound did not induce any brightness change, suggesting the same mechanism as a gel phantom works in living tissues. Our results are promising for use in a noble ultrasound therapy system with high selectivity and safety while improving the throughput of current ultrasound tumor treatment systems.

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1. Introduction

Microbubbles are well-established contrast agents used in ultrasound echography and have mostly been used to visualize vascularity.

Recently, they have become promising tools for site-specific diagnosis and therapy in combination with ultrasound. Eliegara et al. first demonstrated targeted contrast imaging by attaching ligand molecules onto the surface of microbubble contrast agents [1]. In addition to diagnostic purposes, microbubbles could possibly be used as sensitizers for high intensity focused ultrasound (HIFU) therapy, which thermally coagulates diseased tissues, because they have been found to enhance the ultrasonically-induced temperature rise of tissues [2].

However, a serious problem arises when using microbubbles in that they are too big (several microns in diameter) to leak into tissues from the blood vessels when intravenously administered. In general, utilizing targeted drugs also requires ‘incubation time’ after injection to let the drug accumulate at the target and then to be washed out from the other sites, typically for more than one hour. Due to the relatively low stability of microbubbles in bodies compared to other types of drugs ever used, there are fewer bubbles that are usable as HIFU sensitizers than expected.

Our approach, enabling the delivery of contrast agents into tumor tissues and also usable as therapeutic sensitizers, uses phase change nano-droplets (PCND) [3,4]. It is known that when water-insoluble chemical compounds are dispersed in water as particles, their apparent boiling points rise due to a kind of superheating phenomena [3]. Such a state is pseudo stable and can

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change to a more stable state through physical stimulus, resulting in a phase change from liquid to gas. The phase change induces the volume and radius inflation of particles' to about three and one orders of magnitude, respectively. Figure 1 shows B-mode images of murine tumor tissue before and after an exposure of typical phase change ultrasound pulse at a frequency of 5-MHz.

We aim to administer PCND and then change their phase to a gas, and to produce microbubbles only at the targets inside the body. The phase change visualizes whethe the droplets are delivered to the target, and we then can further expose the therapeutic ultrasound, such as HIFU for site-specific treatment. This type of 'on-demand' generation of microbubbles has advantages over administering microbubbles themselves if microbubble generation and therapy are performed using the same transducer. The visualization procedure of on-demand microbubbles gives us two important pieces of information on ultrasound therapy, 1) that a sufficient enough amount of therapy sensitizer accumulates at the target, and 2) which site will be treated. Since the focus of an ultrasound is not easily predicted before exposure in non-homogeneous media like the human body, such information is useful for developing safe and effective types of ultrasound therapy.

We have been investigating the effect of PCND on HIFU therapy. It was suggested that a PCND with a high frequency ultrasound could contribute to lowering the required energy for HIFU therapy. In this study, we investigated the effect of PCND in combination of low frequency ultrasound on accelerating the cavitation induction in vitro. And further we investigated the mechanism that allows the PCND to accelerate the cavitation in vitro and confirmed if the induced cavitation can damage tumor tissues.

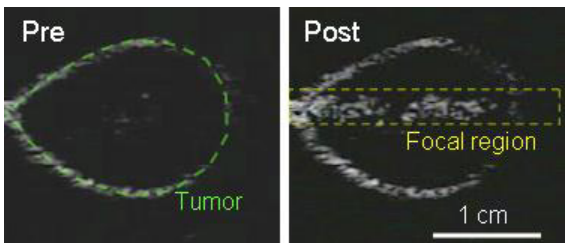

Fig. 1. Typical phase change in tumor tissue

2. Method

PCND and phantom preparation

The PCND preparation procedure has been described elsewhere [4]. In brief, phosphatidyl coline liposome is prepared and the liposome suspension is further emulsified using a high level of pressure (20 MPa) in the presence of perfluorocarbon (perfluoropentane + perfluorohexane) liquids. The size distribution of a PCND was measured using a LB-550 (Horiba, Ltd., Kyoto, Japan) dynamic light-scattering size analyzer. The mean diameter of the PCND was about 0.2 □m. A non-phase-change nano droplet (NPCND) was prepared by replacing the perfluoropantane and perfluorohexnane with pefluorooctane. A gel phantom was prepared using basically the same procedure as previously reported [6].

Experimental setup for ultrasound exposure

In this study, both the in vitro and in vivo experiments were performed using the same setup as shown in Fig. 2. A focused ultrasound transducer (1.1 MHz at a diameter of 48 mm / F number of 1.0) was submerged in a tank of degassed water kept at 37ºC. Gel phantoms with the PCND were placed at the focus of the transducer. Acoustic signals from the gel phantom were received by the focused hydrophone. In vivo experiments were carried out using anesthetized CDF1 mice bearing Colon 26 tumor tissues that were subcutaneously implanted.

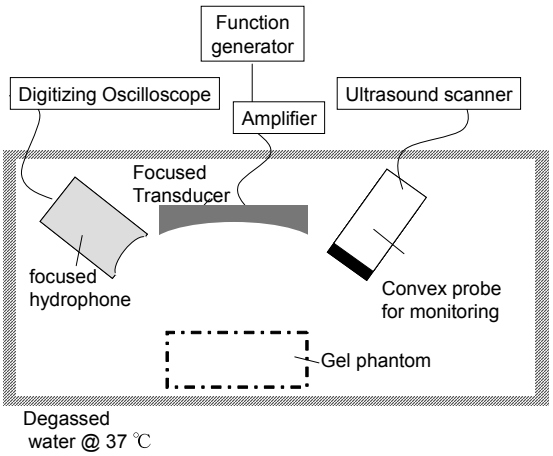

Fig. 2. Experimental setup

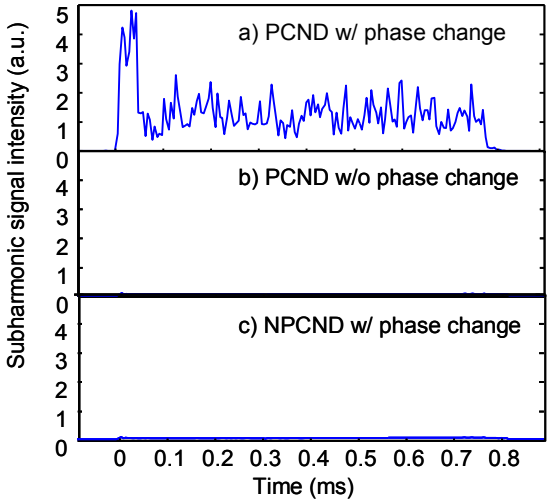

Figure 3 Subharmonic signals from PCND-containing gel phantoms on 1.1 MHz ultrasound exposure

3. Results and Discussion

To investigate the effect of the PCND on accelerating the cavitation induction, we measured the acoustic signals after exposing a 1.1-MHz ultrasound (5 MPa peak negative pressure with 1000 cycles) to a PCND-containing gel phantom with and without the exposure of an ultrasound for the phase change (microbubble generation) of the PCND prior to 1.1-MHz ultrasound. As an ultrasound for the phase change, a 3.3-MHz pulse at an 8.5-MPa peak negative pressure with the duration of 100 cycles was used in this study.

Figure 3 shows the signal intensity of the subharmonic components, which is an indicator of cavitation, obtained during exposure to a 1.1-MHz ultrasound. The results using a NPCND are also shown. In the presence of the PCND, a subharmonic signal was detected only when the phase change ultrasound was exposed. This result suggested that the PCND itself does not work as a cavitation accelerator, but the resulting microbubbles do. The results attained when using a NPCND were received with phase change ultrasound yet no subharmonic signals were observed.

The above results strongly suggest that a PCND works as a cavitation accelerator only when and where a phase change ultrasound is applied and microbubbles are produced. Such a property would lead to a very selective and safe type of tumor therapy using an ultrasound. Other alternative cavitation accelerators for tumor therapy are microbubbles injected as is. Although site-specific microbubbles have already been developed and their accumulation onto particular sites has been confirmed, so far, their effects are limited to use for only diagnostic purposes, presumably due to the small amount of bubbles that accumulate. Non-targeted microbubbles lack selectivity and cannot be an alternative to a PCND.

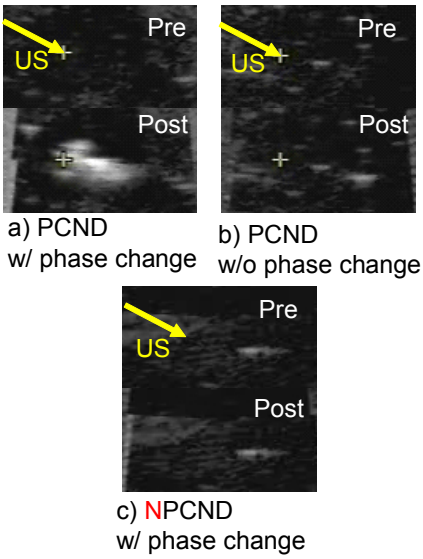

Figure 4 Change of gel phantom in B-mode image on 1-MHz ultrasound exposure

Furthermore, either targeted or non-targeted, the injected microbubbles will not give information on the focus of the therapeutic ultrasound prior to exposure.

The effect of a PCND used as the cavitation accelerator was further investigated from ultrasound diagnostic images. Figure 4 shows the B-images of a PCND-containing gel phantom obtained from the pre and post exposure of a 1.1-MHz ultrasound under the same conditions as shown in Fig. 3 except the number of cycles was increased to 11,000. The exposure was performed with and without the advanced exposure for the phase change. When using the phase-change ultrasound, a clear bright region was created, which was roughly that the size of the focal zone of the 1.1-MHz ultrasound. However, no changes were observed in the absence of the phase-change ultrasound while the same 1.1 MHz ultrasound intensity followed and the PCND existed in the phantom. B-mode images taken for a gel phantom containing NPCND were also shown. No changes were observed.

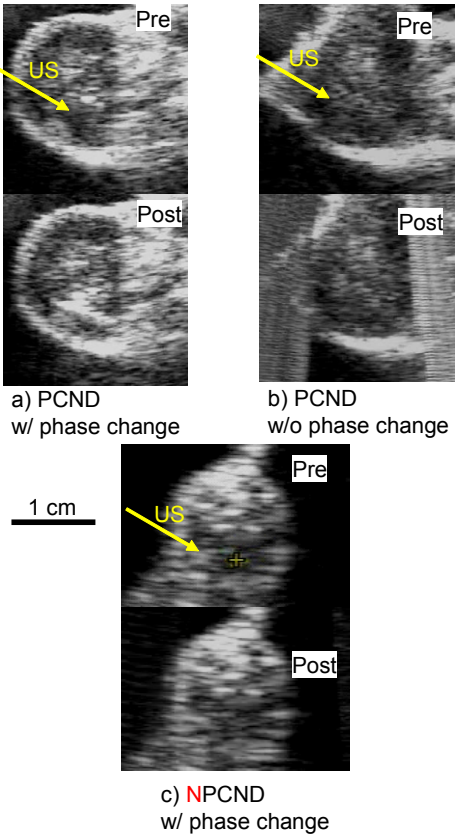

Figure 5 Change of tumor tissue in B-mode image on 1-MHz ultrasound exposure

Taking the results in Fig. 4 into consideration, we presume that when using a PCND and under advanced phase-change ultrasound exposure, a 1.1-MHz ultrasound can generate cavitation at 5 MPa that is visible in ordinal B-mode ultrasound echography. Also

we assume that the non brightness changes in the absence of either the PCND or phase-change ultrasound did not result from the lack in the delectability in cavitation induction, but because no cavitation was induced.

We then proceeded to the in vivo experiments to study whether the above phenomena in vitro also takes place in bodies.

Figure 5 shows B-mode image of colon 26 tumor tissue pre and post ultrasound exposure under the same conditions as shown in Fig. 4. The ultrasound was exposed for 10 s. The tumor tissue was subcutaneously inoculated into a CDF1 mouse. The PCND was administered through the tail vein at a dose of 10 mg/kg. The ultrasound was exposed 5 min after the PCND injection. Similar to what was shown in Fig. 4, a significant amount of brightness change was observed when the phase change ultrasound was applied (Fig. 5a), and was not observed in the presence of the PCND and the absence of phase-change ultrasound (Fig. 5b), and in the presence of the NPCND and the phase-change ultrasound (Fig. 5c). The tendencies in Fig. 5 were the same as those in Fig. 4.

The mouse in Fig. 5a was sacrificed immediately after the experiment and tissue sample was extracted. A cross section of the tissue stained with Hematoxilin-Eosin is shown in Fig. 6. Tissue damage was created at the region where the brightness changes took place in Fig. 5a. In this region, a typical necroses pattern such as deep staining or the elimination of nuclei was observed.

This suggests that the above described cavitation acceleration of the PCND in combination with the phase-change ultrasound also takes place in vivo. Moreover, the visualized cavitation induces tissue damage in tumors.

We concluded that the in vitro and in vivo results match well, and thus, our PCND gives us a way to very selectively induce cavitation at a low acoustic intensity.

In current HIFU tumor therapy systems, relatively low throughput and the potential spatial lag between predetermined and actual ultrasound foci within targets are very serious problems and limit their clinical applications. PCND has a possibility to solve both problems. The throughput problem is mainly due to the necessity of ‘cooling time’ for the tissues in the ultrasound beam path between each ultrasound exposure. Due to the low intensity, (less than 100 kW/cm2 in this study) our system does not seem to require cooling time. This could result in a faster therapy time than conventional HIFU therapy systems. The latter problem could be also solved by our system as follows. With PCNDs and trigger pulses, it would be possible to visualize the focus of the therapeutic ultrasound prior to inducing the cavitation by the phase change of the PCND. If some lags were observed, we could either stop the procedure or re-arrange the focus.

Although further studies are needed on the biological activities of PCND such as diagnostic and therapeutic effects in larger animals or the safety issues of PCND as a medicine, the combination of PCND and ultrasound is very promising as a tool for developing a safe and fast ultrasound therapy system.

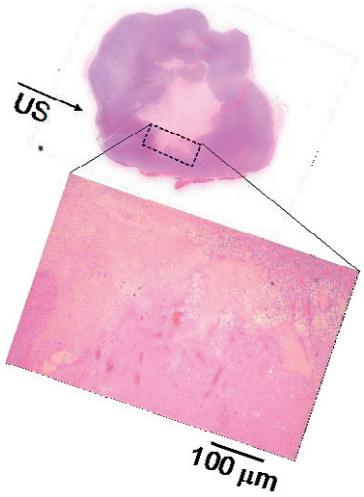

Figure 6 Cross section of tumor tissue exposed to 1 MHz ultrasound in the presence of PCND and phase change ultrasound

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